but this is not sufficient, it seems to me, to justify the association; and the true place for the Cirrhipedes is, as a distinct class, between the Myriapoda and the Annelides. The *Campanulariæ* are not *Acalephæ*, because that, in their embryo state, they have their characters and aspect; nor are the *Acalephæ* members of the order *Anthozoa*, because the young Medusans resemble that order. Such facts indicate an affinity, but do not call for a coalition in one order.

The *Medusæ* are Anthozoans in their young age, and many *Anthozoa* are at first Medusans; hence they necessarily belong to the same class, in which they constitute distinct orders.

The *Hydræ* are not Anthozoans, but rather freshwater *Medusæ*, as I have along ago published. They cannot be separated from each other by any character of importance.

The Tunicata, which more especially occupy our present attention, are very closely connected with the Bryozoa and the Acephala. I have thought it proper to leave them in the class Mollusca, because of the presence more especially of a heart. If they had not that organ, there would have been no more reason to place them amongst the Mollusca than amongst the polypes. In the branch or tribe of the Allo-cotyledones, the first class only, that of the mollusca, possesses a true heart.

XXXIV.—*On the Development of* Chara. By C. Müller*.

[With three Plates.]

§ 1. *Introduction.*

Although this subject has occupied the attention of many observers from an early period, and notwithstanding great light has been thrown upon it by these numerous investigations, still a history of its development combining the diffused observations has hitherto been wanting. My object has been to effect this, and its accomplishment appeared to me the more requisite, inasmuch as this family, which is characterized by so many important structural peculiarities above all other cryptogamic families, nevertheless holds a doubtful position in systematic arrangement. Although from these investigations I do not venture to decide upon its systematic affinities, still, by describing the production of the spores to which so much importance is attached, I hope at least to adduce facts which may bring us nearer the truth. I would willingly have added the development of the anthers at the same time, on which numerous but not conclusive observations have been made; but as the time has arrived at which these

* Translated from the Botanische Zeitung for June 12 and 19, 1845.

organs can be no longer obtained, the substance being destroyed by winter, and as at the next opportunity I may not have the leisure for continuing these researches, I shall present here the observations which I have collected.

As regards the history of this family, we find in Kaulfuss's paper* a complete sketch of it up to that time, to which I must refer; subsequently, Bischoff †, Schultz ‡ and Meyen § have made valuable contributions. Kützing || has detailed some general observations, and Fritzsche some beautiful investigations on the anthers in his paper, "Ueber den Pollen"¶, which however leave several points open for future observations. Nägeli has lately written on the moving spiral fibres in the mucous threads of the anthers**, and Mettenius on the same subject in the 'Bot. Zeitung,' 1845.

Considering the labours of these observers as known, I shall confine myself strictly to my own investigations. But as we have to commence the history of the development with the germ, I do not consider it superfluous again to give a description of the spore in that stage in which it has attained its greatest development (especially as it contains some new facts), because by a knowledge of the organ, in which the formation of the new plant occurs, our knowledge of the process itself must become more perfect.

§ 2. *The Ripe Fruit.*

When divided through the axis it appears composed of three distinct coverings: 1. an external one (the spore-sac); 2. a middle one (the sporular membrane); and 3. an internal one (the nucleus) (Plate V. fig. 1).

The *spore-sac* is a thick, more or less pyriform covering, upon the apex of which five approximated thick cells are situated, forming a kind of crown. This covering is formed of five cells, which are spirally wound around the spores several times—usually twice. The former five short cells form the summit of the spores. Each of these cells when divided appears four-sided, their inner surfaces being flattened towards the spores, the outer ones towards the atmosphere, and the lateral ones towards each other. The planes of the latter however are arranged in an undulatory manner. These, like the former cells, are composed of three distinct membranes; an external one or epidermoidal mem-

* Erfahrungen über das Keimen der Charen, Leipzig, 1825.
† Krypt. Gew. 1 Lief. 1828.
‡ Natur d. lebendigen Pflanze, Bd. 2. p. 470.
§ Physiologie, especially in the third volume.
|| Phycolog. general.
¶ Mém. de l'Acad. Imp. des Sc. de St. Petersbourg.
** Ztschr. f. phys. Bot. Bd. 1. Heft 1. p. 168.

brane*, a secondary, which is easily separated from the latter, and an internal more mucous one, in or upon which the true cellular contents (gonidia of Kützing) are situated. The latter is Mohl's primordial utricle. When treated with nitric acid the entire mass of the secondary membrane separates in an undulatory form (Pl. V. fig. 3). The contents of the cells, which consist of a greenish granular mass, in this stage of the spores are frequently absorbed at several places. The same occurs with the above five terminal cells, the surfaces of which press so closely together that there is no inlet into the spore (Pl. VII. fig. 35). The spore-sac is alone subjected to various alterations in form, the spore always remaining oval.

The *sporular membrane* is likewise a closed covering of a roundish-oval form, with a rounded summit and a truncated base (Pl. V. figs. 1. and 2), which flattens into a cell which will be subsequently considered. It is uniformly thickened, and thence cartilaginous, of a more or less brown colour, and has the same spiral windings as the spore-sac. They correspond to one another with tolerable accuracy, so that they are entirely or very nearly in the same plane. This spiral plane turns from left to right. The five extremities of these thickened, flattened cells of the sporular membrane unite at the apex as in the spore-sac, without forming any appendages. At the highest point the upper portion of the windings projects somewhat at an acute angle (Pl. V. fig. 6).

The *membrane of the nucleus* lies close to the sporular membrane, but quite separate. It of course depends on the form of the sporular membrane, and differs from it merely in its more delicate, transparent, uniform texture, which is neither cellular nor spiral.

The *contents of the nucleus* consist of starch-cells only; these vary in size and are of a more or less rounded, somewhat compressed form. They are perfectly hollow, bursting either lengthwise or in the centre (fig. 5) to discharge their fine granular contents, which also consist only of starch, as shown by the deep blue colour produced by iodine, and which is not unfrequently found between the parent-cells. By gentle pressure I was able to force these contents from the parent-cells, but I have not been able to observe this in loose pieces.

As we have mentioned above, the spore is attached at its base to a *four-sided, tolerably large cell* (figs. 1 and 2). This contains a white, granular, densely aggregated substance, and whilst within the spore-sac might be expected to perform some important part in its nourishment. Moreover in it the nutritious fluid which comes from the stem is rendered assimilable by the nucleus.

* See § 6, where this is compared to the cuticle.

Two other cells, which lie beneath its base and thus flatten it, are also situated within the spore-sac, which surrounds them like a collar. They only form the means of attaching the spores and constitute the direct conductors of the nutritive fluid for the latter. They contain a green mass which is usually spherical. The lowest cell fixes the whole fruit, and to it the spore-sac is also attached between two branches of the stem (Pl. V. figs. 1 and 2).

§ 3. *The Germinating Spore.*

The period of the development of the germ appears to vary in different species. Bischoff * states on this point, that those *Charæ* which mature their fruit in autumn germinate in the mud of their pools in the spring, and that those which ripen earlier germinate in the autumn. I can confirm the latter statement in *Chara vulgaris* and *hispida,* both of which I saw germinating in the October of 1844. This disproves Kützing's remark †, that in our climate no *Charæ* remain through the winter, and that all are propagated in the spring, partly from seeds and partly from buds. Moreover, much depends upon the temperature of the atmosphere, consequently also of the water, although this can hardly completely prevent the occurrence of a vernal germinating process from absence of heat.

However, as soon as the parent plant fulfils its purpose, the development of the fruit, it decays from the disintegration of its parts, and the fruits thus reach the mud of the water, or before this happens spontaneously separate from the cells of the stalk, as in *Chara crinita,* in which the parent plants continue to live for a considerable time afterwards.

After having arrived in this medium, the spore-sac is dissolved from the spores; this is usually caused by decay. The spore is thus exposed to the immediate action of the water, but nevertheless requires a considerable time before it is capable of development.

A simple process ensues within it; for the starch-cells swell from the imbibed water and assume another state of aggregation. They break up into a mucous, oily-looking mass, which is filled with extremely delicate and minute granules. I have observed this disintegration directly and distinctly, as seen in fig. 20. After having looked at the small brown cells for some time under the microscope, and carefully moved them to and fro with a lancet to ascertain their form and size they suddenly became flattened, and in their place there appeared a considerably larger globular mass (fig. 20), which only differed from the former in its uncommonly delicate mucous structure and its much greater transpa-

* *L. c.* p. 8.

† *L. c.* p. 318.

rence. This globule did not deliquesce, was moveable to and fro much as before, and was only rendered slightly brown by iodine, whereupon the above delicate granules became again perceptible, having become coloured somewhat more brown.

Thus the starch-cells enter into direct combination with the elements of the water, as they are now no longer coloured blue, but brownish by iodine. But the bursting leads us to imagine that the softened and metamorphosed starch-cells are inclosed by an extremely delicate membrane, which cannot be again rendered evident, and the existence of which would also indicate that the formative process takes place from within outwards. According to Schleiden it is the reverse; but this appears to me the only deduction on the point. He has also correctly supposed* that the starch gradually becomes *merely* finely divided and not chemically dissolved; for in consequence of the constant existence of the above minute granules, which are here constituted of starch *only*, no further inference can be made. As there is no other substance in the spore than starch, the granules *must* be formed from it. Hence we have *merely* another state of aggregation, from which new modifications, such as cellulose, dextrine, membranous substance and all their isomeric compounds, may be formed, but into which we cannot enter any further.

This sufficiently proves that an apparently oily mucous liquid is formed from the starch, and several observers attest the existence of true globules of oil in it. Whether such really exist in the fertile starch-cells, whether they are formed simultaneously with the starch in a different state of aggregation, or *whether they exist at all*, I must leave undetermined. It is more important that by this formation the starch is prepared so as to be assimilated by the membrane of the nucleus. Hence we may designate the above fluid with good reason and correctly as *cytoblastema*.

As soon as this is assimilated the *above membrane* expands lengthwise, ruptures the sporular membrane at the apex, presses back the five cells of the sporular membrane which confine it like five valves, and thus appears in the form of a simple transparent vesicle, which now only elongates, so as to proceed rapidly towards the formation of the germ-plant. That it is merely the membrane of the nucleus which here expands, may be seen with the greatest certainty—although Bischoff doubts it—in a true nucleus-sac taken out of the sporular membrane (fig. 4).

§ 4. *The Embryo.*

The vesicle now elongates so as to form a utricular cell, the apex of which then speedily becomes spherical, and oblique septa

* Grundz. i. p. 179.

are formed within it, whence it acquires as many subdivisions, parts or cells (fig. 6). These subdivisions are at first of equal length; but as they continue progressively to be developed, the lowermost takes the lead and becomes more elongated. The lower ones are then usually the most transparent; the green contents of the cells are more developed in the upper ones, although they are subsequently formed also in the former, seldom however to the same extent. The elongation of the stem now proceeds simply in the same manner, until finally new cells, the whorl of branches, are likewise formed from its subdivisions. These are either developed, as is usually the case, in the earlier stage of the development of the stem *on one side only,* or subsequently in *a complete whorl.* Moreover new cells, i. e. *shoots,* are formed from their joints according to the same laws as from the stem, for there can be no question here of any development of leaf.

As soon as the stem contains a few cells in its interior, a remarkably higher development ensues from its lowermost subdivisions. The whorls do not stop short at the formation of shoots as in the upper portions, but are developed into *new plants* (figs. 6, 7, 8 and 10). This formation is a perfect repetition of the development of the nucleary membrane. As in it, the knotty-looking cells at the joints (fig. 7) elongate in a sacciform manner (fig. 8), their apices at the same time becoming enlarged (fig. 10); these again form new cells in their interior; the lowest appear more transparent, whilst in the uppermost the green cellular contents are formed. At the base of the axis, close above the orifice of the spore, a similar cell-formation has occurred. For as soon as the nucleary membrane began to burst through the sporular membrane like a bladder and to expand it in a sacciform manner, it began to be developed in a sacciform manner on the opposite side (figs. 4 and 6). Thus the spore, which is at first perpendicular, acquires a horizontal position. Each utricle forms a rootlet, and others follow it from simple vesicular expansion of the nucleary membrane, so that it acquires, at this end, a complete head of root-fibrils.

The question now is—*how are all these cells formed*? The direct answer is—*by cytoblasts and by these alone.* I have not been able to observe this so distinctly in the earliest cells of the stem, although there can be no doubt about it, because there is not the least appearance of a secondary membrane, which might perhaps have become contracted by the well-known process of division, and thus formed new cells. But in the formation of the branches and of the new plants from them, the whole process may be traced most unequivocally.

The cytoblastema, or the above-described liquid which is formed from the starch, possesses the remarkable peculiarity that it is very readily formed into globular masses. This may be very easily seen by the action of iodine, in the lowest simple elongation of the nucleary membrane (figs. 7 and 8). This lowermost portion of the stem is always filled with the cytoblastema as far as the first internal cell, whilst the rootlets are separated at their base from the interior of the nucleus by septa which have likewise originated from cytoblasts by the formation of cells, so that it empties its contents at once into the former subdivision.

The ready tendency to spherical aggregation of the cytoblastema favours the formation of the cytoblasts, or rather constitutes their very commencement. When this fluid cytoblastema is taken up endosmotically by the cells, we see how it is also deposited between every two septa of the cells; hence in each segment of the stem (fig. 7), separate globular masses are soon formed in the very substance of the cytoblastema with which the formation of the cytoblasts commenced. Increasing rapidly in circumference, their outline appears sharper, and in a short time they become so swollen as to protrude the cell-membrane of the axis externally, in the form of a bladder. The process continues until the vesicular projections have become cylindrical (fig. 8). It then ceases in the branches, between the articulations of which the same process subsequently commences for the formation of the shoots. On the further evolution of these to form new plants the cells become utricular (figs. 6, 10). Finally a new cell-formation commences in them by cytoblasts, as we may certainly suppose to happen in the main stem, since in this case it is merely a simple repetition of its formation. By this new cell-formation the apex of a utricular cell is protruded like a knob (fig. 1, a very early stage !), and we have a new stem presented to our view, which is capable, like the main stem, of further development. This property of the plants, to form new individuals by *intercalary* growth, explains the great power of diffusion of the *Charæ,* which is so considerable, that when a *Chara* has been removed from the water we cannot determine in most cases its true point of attachment to the soil. We have a large number of separate plants on a single plant.

In fig. 9 we have the complete process of cell-formation before us. The two upper cells are separated from the membrane of the stem by the action of iodine. The remains of the cytoblasts are still distinctly perceptible at the walls. Here and in fig. 7, mucilaginous threads arise from them, and are diffused in the form of a web throughout the cells, as we frequently see in Algæ. On account of the great transparency of the object (fig. 9), it could not be ascertained whether currents occurred in the sap; these threads cannot be confounded with them, as they are coloured brown by iodine. Generally speaking this can rarely be observed in *Chara*, because the cells become opake too soon and rarely permit of their contents being distinguished, as they are rapidly deposited on the walls of their cells. One circumstance however remains inexplicable, which is, that in one cell (fig. 9) there are cytoblasts which appear as if they were divided into two parts; one in the third upper cell still with its apex in the third, the greater part being in the upper one (had the cytoblast, which was formed in the third cell, really ruptured this to form a new cell?); and lastly, that the cytoblast of the uppermost cell, as also the cell itself, appeared as if contracted exactly at the centre!

The membrane of the new cells, which is formed in an annular manner by the cytoblasts (this is also Schleiden's view), is Mohl's primordial utricle*. It can hardly be seen more beautifully *in situ* in other parts than in the lowermost joint of the stem of the germinating plant (figs. 7 and 8). It is here a simple induration of that external lamina of the cytoblasts which lies immediately on the internal periphery of the stem membrane. The membrane which surrounds the cytoblast is perfectly analogous to that produced by induration. Both are coloured yellowish or brownish by iodine. Hence Mohl is correct, when he says†, "The substance of which the primordial utricle consists, appears, if not identical with, at least nearly related to the mucous granular substance which usually envelopes the nucleus in the form of an irregular mass, and from which the mucous threads which are so frequently met with in the young cells emanate, since these different portions react in the same manner with iodine and sulphuric acid." We ought to consider them, as stated above, to be identical. The same author's opinion also ‡, that the primordial utricle might be of a nitrogenous nature, considering the above reaction of iodine and sulphuric acid, the latter of which does not cause it to disappear, regarded by French chemists as a proof of its containing nitrogen, appears susceptible of direct proof from the above facts. For if the formation of the cytoblastema from starch and that of the cytoblasts and primordial utricle from the former can be directly observed, the conclusion regarding nitrogen is not too bold, if we remember the large amount of nitrogen contained in the gluten of starch. And starch is always found naturally combined with this substance!

When the primordial utricle is once perfectly formed, it appears as a transparent, completely closed peripheral membrane, which is entirely separated by iodine from the outer cell-membrane and envelopes the cell-contents. It adheres to it in the upper cells equally as strongly as we previously found it do in

* Grundzug, 2te Ausg. † Bot. Zeit. 1844, p. 244. ‡ *L. c.* p. 305.

the utricular cells of the spore-sac. The primordial utricle is however completely isolated in the lower cell, *i. e.* the immediate elongation of the nucleary membrane itself, the contents of which do not consist as before of chlorophylle, but of cytoblastema. When acted upon by nitric acid, it frequently contracts so much, as to appear torn into large band-like fragments, which become somewhat spirally twisted (fig. 25). Its membrane is not perfectly smooth but finely granular. This does not occur so much in the lowest segments of the stem. It is remarkable, that when treated with nitric acid, which contracts it considerably, it exhibits various-sized conical prominences. Hence it appears somewhat angular or wavy. The small projections appear generally to pass into minute depressions on the axial membrane. At a subsequent period a secondary membrane is formed between it and the axis.

From what has been stated, it appears that the stem consists of an epidermoidal membrane, subsequently also of a secondary one, the primordial utricle and the cell-contents. The above epidermoidal membrane, which is the direct elongation of the nucleary membrane, *continues to grow with the plant, and in such a manner that the plant remains in it as in a bag.* Kützing calls this membrane the *peridermis,* and considers it as identical with the cuticle of Brongniart, which covers the true epidermis of more highly organized plants *. There can however be no question here of a true epidermis, nor indeed in any of the Algæ.

[To be continued.]

XXXV.—*On the Occurrence of Tetraspores in Algæ.* By G. H. K. Thwaites, Esq.

To the Editors of the Annals of Natural History.

Gentlemen, 2 Kingsdown Parade, Bristol, March 19, 1846.

In the last December Number of your valuable Journal is an extract from a letter presented by M. Montagne to the French Academy on the subject of an interesting Alga belonging to the *Zygnemata,* and discovered by M. Durieu in Algiers, in which the fruit consists of four distinct spores in each sporangium.

The Rev. M. J. Berkeley obligingly favoured me with a sight of an authentic specimen of this species, in which the character was very obvious.

On examining, a few days ago, some spores of *Mesocarpus scalaris,* Hassall, I thought I could detect in them indications of a quaternary division, and I sent specimens to Mr. Berkeley for

* Kützing, *l. c.* p. 86.